THE DISTRIBUTION OF FORMIC AND ALCOHOL DEHYDROGENASES IN THE HIGHER PLANTS, WITH PARTICULAR REFERENCE TO THEIR VARIATION IN THE PEA PLANT DURING ITS LIFE CYCLE.

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(Nine Text-figures.)

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Introduction.

In the study of any enzyme it becomes necessary to obtain some idea of its distribution in nature. So it was that the present investigation arose from a study of the formic dehydrogenase system of pea seeds. This dehydrogenase has only been reported in the seeds of pea and the French or Kidney bean so that the problem to be attacked was whether it occurred in other plants and if so was there any generic significance in its distribution. As a companion study the distribution of alcohol dehydrogenase has also been investigated.

Thunberg (1921, 1936*a*) first discovered a formic dehydrogenase in the seeds of *Phaseolus vulgaris* (French bean). The same enzyme, obtained from peas by Fodor and Frankenthal (1930), was found to differ from the formic dehydrogenase of bacteria in that it required a coenzyme which was present in boiled pea juice and, to a lesser extent, in boiled yeast juice. Andersson (1934) and Lichtenstein (1936) were able to identify this factor as coenzyme I. In 1937 Adler and Sreenivasaya, again using peas, studied the formic dehydrogenase system in detail. They drew attention to the strong inhibition obtained with small amounts of cyanide, a property differentiating this enzyme from all hitherto known cozymase-requiring dehydrogenases.

The alcohol dehydrogenase of plants is much better known than is formic dehydrogenase and although alcohol does not seem to be readily oxidized in some plants (Thomas and Fidler, 1941), the presence of an active alcohol dehydrogenase has been reported from many sources. Thunberg (1921, 1929, 1936*b*) found the enzyme in the following species: *Phaseolus vulgaris* (French bean), *Citrus aurantium* (orange), *Corchorus capsularis* (jute), *Cucumis sativus* (cucumber), *Echinocystic lobata* (a climbing herb), *Evonymus europaeus* (spindle-tree), *Mucuna utilus*, *Pistachia vera*, *Pisum sativum* (green pea) and *Poinciana regia*. Andersson (1933) mentions this enzyme as one of a number of cozymase-requiring dehydrogenases found in cucumber seeds. Adler and Sreenivasaya (1937) studied the alcohol dehydrogenase of pea seeds and determined its pH optimum and equilibrium constant. Some later work by Berger and Avery (1943) includes a study of alcohol dehydrogenase as it occurs in the *Avena* coleoptile.

These references are by no means exhaustive but point to the relatively widespread interest in alcohol dehydrogenase as a distinct contrast to the lack of attention to formic dehydrogenase by plant biochemists.

Material.

(*a*) *Seeds.*—Most of the seeds used in these studies were obtained from the National Herbarium, Sydney. Pea seeds (unless fresh peas are specified) were commercial household dried peas bought in 1 lb. packets. The dehydrogenase content was independent of the brand, several local brands being used.

(*b*) *Pea Plants.*—These were grown in a normal garden plot between November and February. They were always used within two hours of gathering.

Methods.

Preparation of Extract.—For the preparation of materials used in testing for formic and alcohol dehydrogenases a standard procedure was adopted. Seeds were soaked in distilled water overnight. Often it was necessary to soften those with hard testas either by putting in boiling water or by mechanical cracking before soaking. The material was frozen before extraction but otherwise the preparations were made at room temperature. All plant materials were homogenized by mincing and/or grinding in a mortar with sand depending on the procedure required to produce a fine state of subdivision. Water was added in small quantities, the amount depending on the material—usually twice or three times the dry weight of the seeds; generally none was needed with other plant material, the cell sap providing the necessary consistency.

Homogenates were squeezed through muslin and the extract centrifuged and/or filtered through kieselguhr. The filtrate, which was examined immediately, was usually moderately clear and colourless so that the decolorization of the redox dye was easy to follow.

Thunberg Technique.—In studying the distribution of formic and alcohol dehydrogenases in plants the Thunberg technique was invariably used. The contents of the main part of the tube were: 1 ml. extract, 0·6 ml. 0·2 M phosphate buffer pH 7·0, 0·2 ml. coenzyme I solution (60μg.), 0·3 ml. substrate or water. In the hollow stopper was 0·3 ml. 0·0025 M thionine. These concentrations of reactants are based on the series of experiments outlined below which aimed to find the optimum conditions. The tubes were evacuated three times by means of a water aspirator and refilled with nitrogen gas freed of oxygen by passing over heated copper filings. The tubes were then closed and incubated at 38°·C. for 10 minutes before the dye was tipped into the reaction mixture. The times taken for decolorization of the dye were noted and the time of the blank containing water was compared with those taken for the tubes containing alcohol and formate.

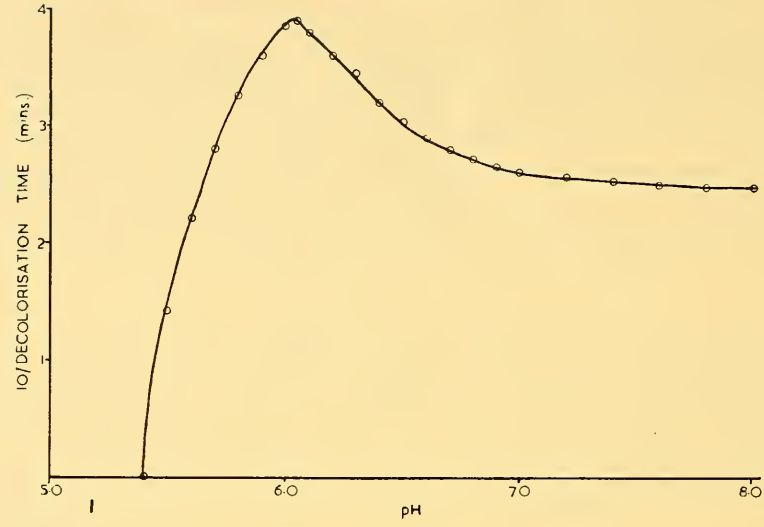
Buffer.—The buffer used had a final concentration of M/20, a concentration which seems to be most suited for plant work, from frequent mention in the literature. Adler and Sreenivasaya (1937) state that the optimum pH for formic dehydrogenase lies between 5·5 and 6·0. Using both 0·5 M acetate and 0·2 M phosphate buffers the formic dehydrogenase system has been tested in this laboratory at all hydrogen ion concentrations from pH 4·4 to pH 8·0. The pH of each reaction mixture was checked at the conclusion of the experiment and found to agree very closely with the pH value of the buffer used. A Leeds and Northrup portable glass electrode was used for these determinations. The optimum pH was found to be pH 6·0–6·1 (Text-fig. 1). Below pH 5·3 no reduction of methylene blue could be obtained and precipitation generally occurred. On the acid side a sharp drop from the optimum is seen while the activity decreases more slowly towards the neutral point and in alkaline reaction up to about pH 8·2 it remains constant. It is noteworthy in connection with the type of buffer used that this anaerobic system was just as active in borate, phthalate and acetate buffers as in phosphate buffers of the same pH values. In these experiments as well as those in the following sections an ammonium sulphate precipitate was used in order to reduce the blank and obtain more clear-cut optima. The peas are soaked overnight in distilled water. The soaked peas are homogenized under nitrogen in a Waring Blendor for two minutes with twice their dry weight of distilled water. The homogenate is squeezed through muslin and the extract centrifuged for ten minutes. The supernatant is half-saturated with ammonium sulphate and the precipitate taken up in distilled water, equal to half the amount used for extraction, and dialysed overnight in the refrigerator against a large volume of distilled water. The dialysate is centrifuged for five minutes and the supernatant is the enzyme preparation referred to in Text-figures 1, 2, 3 and 4.

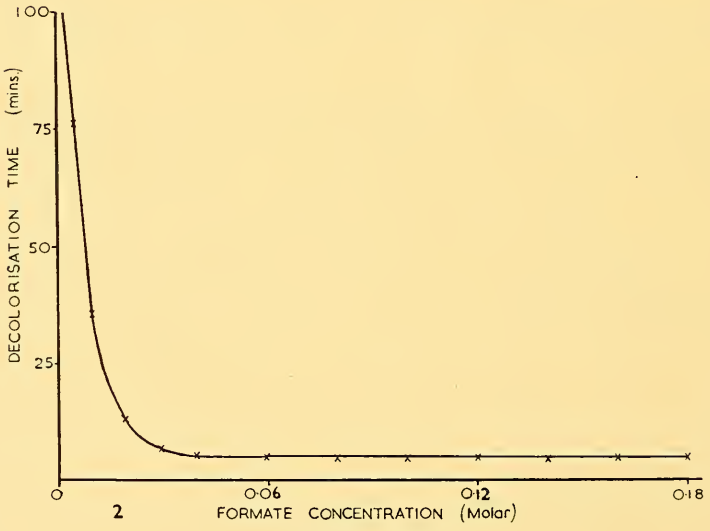
The optimum pH of alcohol dehydrogenase is between 7·8 and 8·3 (Berger and Avery, 1943). For these experiments then pH 7·0 was chosen as it lies mid-way between the optima of the two dehydrogenases, and both enzymes work well at that reaction.

Substrate.—The concentration of formate used (0·3 ml. 0·2 M sodium formate) was optimum as decided from experiments with pea extract purified by half-saturation with

ammonium sulphate as above (Text-fig. 2). Further increase of the formate concentration from the optimum of 0·025 M to 0·18 M has no effect.

The rather high final concentration of alcohol was chosen to allow for loss of substrate during the evacuation and gassing of tubes.





Text-figures 1, 2.

1.—The effect of pH on the decolorization of methylene blue by the formic dehydrogenase system of peas. Thunberg contents: 0·5 ml. enzyme preparation, 0·2 ml. coenzyme I (60μg.), 0·3 ml. 0·2 M sodium formate, 0·5 ml. buffer of various pH values, 0·2 ml. 0·025% methylene blue.

2.—Formate concentration curve. Thunberg contents: 0·5 ml. pea enzyme preparation, 0·5 ml. 0·2 M phosphate buffer pH 6·0, 0·2 ml. coenzyme I (60μg.), 0·2 ml. 0·025% methylene blue. Total volume 2·3 ml.

Coenzyme I.—This substance was prepared by the method of Williamson and Green (1940), and although purity was only about 6·0% as determined by spectrophotometric measurements of the absorption of the dihydroderivative at 340mμ, nevertheless the activity of the preparation in the formate and alcohol systems was very high. The amount used (60μg.) was optimal as decided from Text-figure 3 where the amounts of coenzyme stated are the amounts actually present, 6·0% purity being allowed for.

The reversibly oxidizable Dye.—Theoretically any reversible indicator whose potential is more positive than that of the formate-bicarbonate and alcohol-acetaldehyde systems would be reducible by these two dehydrogenase systems. From a consideration of E'_0 values it is probable that more negative dyes would have been more suitable for the study of these systems but the choice was limited to methylene blue ($E'_0 + 0{\cdot}01$ volt) or thionine (Lauth's violet; $E'_0 + 0{\cdot}063$ volt). That thionine is the more efficient of the two is seen from Text-figure 4. This is particularly the case with alcohol dehydrogenase.

TABLE 1.

Distribution of Formic and Alcohol Dehydrogenases in the Seeds of Gymnosperms.

Order and Family.	Name of Genus and Species.	Common Name.	Decolorization Times.				
			Blank.	Formate.		Alcohol.	
GINKGOALES—							
Ginkgoaceae ..	*Ginkgo biloba.*		29½ m.	12½ m.	++	25 m	+
CONIFERAE—							
Pinaceae ..	*Callitris calcarata.*	Black Murray Pine.	20 hrs.	20 hrs.	–	20 hrs.	–
Pinaceae ..	*Callitris cupressiformis.*	Victorian Cypress.	22 hrs.	21 hrs.	–	21 hrs.	–

TABLE 2.

Distribution of Formic and Alcohol Dehydrogenases in the Seeds of Monocotyledons.

Order and Family.	Name of Genus and Species.	Common Name.	Decolorization Times.				
			Blank.	Formate.		Alcohol.	
GLUMIFLORAE							
Graminae ..	*Triticum aegilopoides.*	Wheat.	14 m.	18 m.	–	4 m.	++
LILIFLORAE—							
Amaryllidaceae	*Doranthes Palmeri.*	Palmer's Spear Lily.	12 hrs.	10½ hrs.	?	6½ hrs.	+
Liliaceae ..	*Smilax glycyphylla.*	N.S.W. Sarsaparilla.	10 hrs.	10½ hrs.	–	9½ hrs.	–
SCITAMINEAE—							
Cannaceae ..	*Canna* spp.	Canna.	>4 hrs.	130 m.	+	10½ m.	++

TABLE 3.

Distribution of Formic and Alcohol Dehydrogenases in the Seeds of Dicotyledons.

Order and Family.	Name of Genus and Species.	Common Name.	Decolorization Times.				
			Blank.	Formate.		Alcohol.	
URTICIFLORAE							
Moraceae ..	*Ficus Bellingeri.*	Bellinger River Fig Tree.	62 m.	57 m.	+	15 m.	++
Urticaceae ..	*Urtica dioica.*	Stinging Nettle.	50 m.	43 m.	+	45 m.	+
PROTEALES—							
Proteaceae ..	*Grevillea Hilliana.*	White Silky Oak.	53 m.	10½ m.	++	3½ m.	++
Proteaceae ..	*Macadamia ternifolia.*	Queensland Nut-tree.	88 m.	23 m.	++	4 m.	++
Proteaceae ..	*Stenocarpus sinuatus.*	Queensland Tulip Tree.	120 m.	120 m.	–	62 m.	+
Proteaceae ..	*Telopea speciossima.*	Waratah.	31 m.	10½ m.	++	2 m.	++
SANTALALES—							
Santalaceae ..	*Fusanus acuminatus.*	Quandong.	40 m.	17 m.	++	2 m.	++

TABLE 3.—*Continued.*

Order and Family.	Name of Genus and Species.	Common Name.	Decolorization Times.				
			Blank.	Formate.		Alcohol.	
CENTRO-SPERMAE—							
Chenopodiaceae	*Atriplex spongiosum.*	Spongy Salt Bush.	10 hrs.	105 m.	+	5 m.	++
RANALES—							
Lauraceae ..	*Cryptocarya oborata.*	Obovate - leaved Australian Nutmeg Laurel.	16 hrs.	18 hrs.	–	18 hrs.	–
Lauraceae ..	*Cryptocarya triplinerris.*	Three-nerved Australian Nutmeg Laurel.	16 hrs.	18 hrs.	–	18 hrs.	–
Ranunculaceae	*Clematis aristata.*	Australian Virgin's Bower.	20 hrs.	20 hrs.	–	20 hrs.	–
RHOEADALES—							
Capparidaceae	*Capparis nobilis.*	Caper Orange Tree.	4½ hrs.	65 m.	++	3 hrs.	++
Cruciferae ..	*Brassica oleracea* (var. *capitata*).	Cabbage.	16 hrs.	16 hrs.	–	16 hrs.	–
	B. oleracea (var. *gemnifera*).	Brussels sprouts.	16 hrs.	16 hrs.	–	16 hrs.	–
	B. oleracea (var. *botrytis*).	Broccoli.	16 hrs.	16 hrs.	–	16 hrs.	–
	B. oleracea (var. *boyrytis*).	Cauliflower.	17 hrs.	17 hrs.	–	17 hrs.	–
	B. rapa.	Turnip.	60 m.	50 m.	+	25 m.	++
	Rasphanus sativus.	Radish.	55 m.	66 m.	–	20 m.	++
PARIETALES—							
Passifloraceae ..	*Passiflora edulis.*	Passionfruit.	>150 m.	120 m.	+	19 m.	++
CUCURBITALES							
Cucurbitaceae	*Cucurbito pepo.*	Pumpkin.	29 m.	22 m.	+	43 sec.	++
Cucurbitaceae	*Cucurbita pepo.*	Squash.	23 m.	20 m.	–	1 m. 10 sec.	++
Cucurbitaceae	*Cucurbito melo.*	Honeydew Melon.	29 m.	35 m.	–	3½ m.	++
Cucurbitaceae	*Cucurbita melo.*	Rock Melon.	15 m.	14 m.	–	1 m. 10 sec.	++
MALVALES—							
Elacocarpaceae	*Elaeocarpus cyaneus.*	Pink-flowered Olive-Berry Tree.	5 hrs.	5 hrs.	–	4¾ hrs.	–
Sterculiaceae ..	*Brachychiton discolor.*		2½ m.	60 sec.	++	30 sec.	++
GERANIALES or RUTALES—							
Rutaceae ..	*Boronia Barkeriana.*	Barker's Boronia.	18 hrs.	17 hrs.	?	19 hrs.	–
Simarubaceae ..	*Guilfoylia monostylis.*	Yellow - flowered Pyramid Tree.	10 hrs.	9 hrs.	–	60 m.	++
Meliaceae ..	*Synoum glandulosum.*	Spurious Rosewood.	170 m.	170 m.	–	170 m.	–
SAPINDALES—							
Akaniaceae ..	*Akania Hillii.*	Hill's Horse-Radish Tree.	225 m.	240 m.	–	10 m.	++
Sapindaceae ..	*Alectryon subcinereus.*		20 hrs.	21 hrs.	–	20 hrs.	–
Sapindaceae ..	*Cupariopsis anacardiodes.*	Carrot Wood.	20 hrs.	19 hrs.	?	20 hrs.	–
Sapindaceae ..	*Harpullia pendula.*	Moreton Bay Tulip Wood.	12 hrs.	12 hrs.	–	12 hrs.	–
Sapindaceae ..	*Dodenaea viscosa.*	Victorian Lignum vitae.	60 m.	11 m.	++	3 m.	++
CELASTRALES—							
Celastraceae ..	*Celastrus australis.*	Australian Staff Vine.	10 hrs.	150 m.	++	22 m.	++
Celastraceae ..	*Elaeodendron australe.*	Australian Scarlet-fruited Olive Plum.	225 m.	180 m.	+	19 m.	++
ROSALES—							
Pittosporaceae	*Pittosporum phyllyraeoides.*	Butter-bush.	19 hrs.	20 hrs.	–	20 hrs.	–
Pittosporaceae	*Pittosporum revolutum.*	Yellow - flowered Brisbane Laurel.	6 hrs.	6 hrs.	–	4 hrs.	?+
Rosaceae ..	*Pyrus malus.*	Apple.	75 m.	20 m.	++	14 m.	++

TABLE 3.—*Continued.*

Order and Family.	Name of Genus and Species.	Common Name.	Decolorization Times.				
			Blank.	Formate.		Alcohol.	
Leguminosae ..	*Acacia elata.*	Cedar Wattle.	74 m.	30 m.	++	8½ m.	++
Leguminosae ..	*Acacia hakeoides.*	Black Wattle.	66 m.	35 m.	+	17 m.	++
Leguminosae ..	*Acacia mollissima.*		50 m.	43 m.	+	8½ m.	++
Leguminosae ..	*Cassia corymbosa.*		145 m.	90 m.	+	25 m.	++
Leguminosae ..	*Cassia didymobotrya.*		55 m.	20 m.	++	2¼ m.	++
Leguminosae ..	*Cassia eremophila.*	Desert Cassia.	70 m.	40 m.	+	10 m.	++
Leguminosae ..	*Cassia laevigata.*	Golden Glory Bush.	120 m.	45 m.	++	15½ m.	++
Leguminosae ..	*Chorizema cordatum.*	Flame Pea Bush.	3 hrs.	70 m.	+	40 m.	++
Leguminosae ..	*Caesalpinia Gillesci.*		60 m.	6½ m.	++	5 m.	++
Leguminosae ..	*Dillwynia brunioides.*		6 hrs.	64 m.	++	34 m.	++
Leguminosae ..	*Erythrina crista-galli.*		50 m.	1½ m.	++	2½ m.	++
Leguminosae ..	*Genista alba.*		3 hrs.	70 m.	+	23 m.	++
Leguminosae ..	*Hardenbergia coccinea.*		>330 m.	57 m.	++	50 m.	++
Leguminosae ..	*Hardenbergia ovata-rosa.*		300 m.	120 m.	+	110 m.	+
Leguminosae ..	*Kennedya rubicunda.*	Red Bean Flower.	>300 m.	63 m.	++	33 m.	++
Leguminosae ..	*Phaseolus caracalla.*		>300 m.	95 m.	++	23 m.	++
Leguminosae ..	*Phaseolus vulgaris.*	French Bean.	4 m.	1 m. 20 sec.	++	55 sec.	++
Leguminosae ..	*Pultenaeae flexilis.*	Flexible Pultenaea.	2½ hrs.	80 m.	+	17 m.	++
Leguminosae ..	*Leucena glauca.*		>300 m.	190 m.	+	22 m.	++
Leguminosae ..	*Spartium junceum.*	Spanish Broom.	195 m.	38 m.	++	8½ m.	++
Leguminosae ..	*Swainsona coronillifolia.*		>280 m.	190 m.	+	220 m.	+
Leguminosae ..	*Viminaria denudata.*	Australian Bush Broom.	>280 m.	220 m.	+	*ca.* 280 m.	?
Leguminosae ..	*Sesbania tripellii.*		>3 hrs.	65 m.	++	13 m.	++
Leguminosae ..	*Vicia faba.*	Broad Bean.	3 m.	2 m.	+	1 m. 20 sec.	++
Leguminosae ..	*Pisum sativum.*	Pea.	5 m.	1 m. 10 sec.	++	1 m. 15 sec.	++
Leguminosae ..	*Glycine soja.*	Soya bean.	160 m.	135 m.	+	145 m.	+
Leguminosae ..	*Trifolium repens.*	White Dutch Clover.	180 m.	65 m.	+	70 m.	+
Leguminosae ..	*Trifolium subterraneanum.*	Subterranean Clover.	55 m.	30 m.	+	32 m.	+
MYRTIFLORAE							
Thymelacaceae	*Phaleria Neumanni.*		12 hrs.	9 hrs.	+	8½ hrs.	+
Myrtaceae ..	*Tristania laurina.*	Turpentine Box.	16 hrs.	16 hrs.	−	13 hrs.	+
UMBELLIFLORAE—							
Araliaceae ..	*Tieghemopanax elegans.*		16 hrs	15½ hrs.	−	15½ hrs.	−
Umbelliferae ..	*Daucus carota.*	Carrot.	5¼ hrs.	5¼ hrs.	−	5¼ hrs.	−
Umbelliferae ..	*Pastinaca sativa.*	Parsnip.	5 hrs.	5 hrs.	−	4¼ hrs.	−
Umbelliferae ..	*Petioselinum hortense.*	Parsley.	4½ hrs.	4½ hrs.	−	4¾ hrs.	−
EBENALES—							
Ebenaceae ..	*Diospyros pentamera.*	Black Myrtle Plum.	49 m.	27 m.	+	37 m.	+
CONTORTAE—							
Apocynaceae ..	*Carissa orata.*	Black Lime Bush.	20 hrs.	16 hrs.	+	15 hrs.	+
TUBIFLORAE—							
Verbenaceae ..	*Vitex acuminata.*		2½ hrs.	2¼ hrs.	−	2½ hrs.	−
Solanaceae ..	*Solanum lycopersicum.*	Tomato.	100 m.	47 m.	+	2½ m.	++
PLANTAGINALES							
Rubiaceae ..	*Hodgkinsonia ovatiflora.*		6 hrs.	5½ hrs.	−	6 hrs.	−
CAMPANULALES							
Goodeniaceae ..	*Goodenia paniculata.*	Panicle - flowered Goodenia.	>16 hrs.	6 hrs.	+	110 m.	++
Compositae ..	*Cichorium endivia.*	Endive.	4½ hrs.	4½ hrs.	−	30 m.	++
Compositae ..	*Lactuca sativa.*	Lettuce.	4⅘ hrs.	4 hrs.	−	25 m.	++

TABLE 4.

Distribution of Formic and Alcohol Dehydrogenases in Pisum sativum *during its Life Cycle.*

Age of Seedling. (Days.)	Part of Seedling.	Decolorization Times.				
		Blank.	Formate.		Alcohol.	
0	Mature seed	5 m.	1 m. 10 s.	++++	1 m. 15 s.	++++
1	Residual seed	5½ m.	1 m.	++++	50 s.	++++
	Testa	>60 m.	>60 m.	−	>60 m.	−
	Embryo	7 m.	1½ m.	++++	1 m. 10 s.	++++
	Whole seedling	5½ m.	1 m. 5 s.	++++	55 s.	++++
2	Whole seedling	6 m.	1 m. 15 s.	++++	1 m. 50 s.	+++(+)
3	Residual seed	6½ m.	1¾ m.	+++	1 m. 35 s.	+++
	Testa	>150 m.	>150 m.	−	>150 m.	−
	Embryo	6 m.	2½ m.	+++	3 m.	++
	Whole seedling	6¼ m.	2½ m.	+++	4¼ m.	++
4	Whole seedling	6½ m.	3 m.	+++	4½ m.	++
5	Residual seed	7 m. 10 s.	2 m. 40 s.	+++	3 m. 10 s.	+++
	Radicle	5 m.	2 m.	+++	5 m.	−
	Plumule	7 m.	2¾ m.	+++	6 m.	+
6	Residual seed	8 m. 10 s.	3½ m.	+++	4 m.	+++
	Radicle	5 m. 50 s.	3 m.	++	5¾ m.	−
	Plumule	10 m.	6 m.	++	8 m.	+
	Whole seedling	7½ m.	4½ m.	++	7 m.	+
7	Residual seed	9½ m.	4 m. 10 s.	+++	5 m. 10 s.	++
	Radicle	8 m.	8 m.	−	8 m.	−
	Plumule	20 m.	17 m.	+	15 m.	+
14	Residual seed	52 m.	39 m.	++	50 m.	−
	Root	15 m.	15 m.	−	15 m.	−
	Shoot	31 m.	23 m.	+	27 m.	+
21	Residual seed	>5 hrs.	>5 hrs.	−	>5 hrs.	−
	Root	27 m.	25 m.	+	24 m.	+
	Shoot	39 m.	35 m.	+	30 m.	+
	Nodules	40 m.	40 m.	−	35 m.	+
28	Root	30 m.	27 m.	+	24 m.	+
	Shoot	45 m.	40 m.	+	36 m.	+
	Nodules	8 m.	6 m.	+	45 s.	++++
35	Root	38 m.	35 m.	+	21 m.	++
	Shoot	50 m.	47 m.	+	42 m.	+
	Nodules	5 m.	3 m.	++	1 m. 10 s.	++++
63	Root	80 m.	50 m.	++	25 m.	+++
	Nodules	7 m.	4 m.	++	1½ m.	++++
	Leaves	70 m.	59 m.	+	49 m.	++
	Stems	52 m.	44 m.	+	38 m.	++
	Whole shoot	65 m.	52 m.	+	45 m.	++
	Flowers and buds	27 m.	18 m.	++	25 m.	+
63	Pods 1·5 cm. long	30 m.	18 m.	++	18 m.	++
	Pods 2·0–3·5 cm.	35 m.	16 m.	+++	13 m.	+++
	Pods 3·5–6·0 cm.	50 m.	12 m.	++++	8 m.	++++
	Very young seeds	17 m.	10 m.	++	4½ m.	+++
77	Seeds from full pod	12 m.	3 m.	++++	1½ m.	++++

RESULTS.

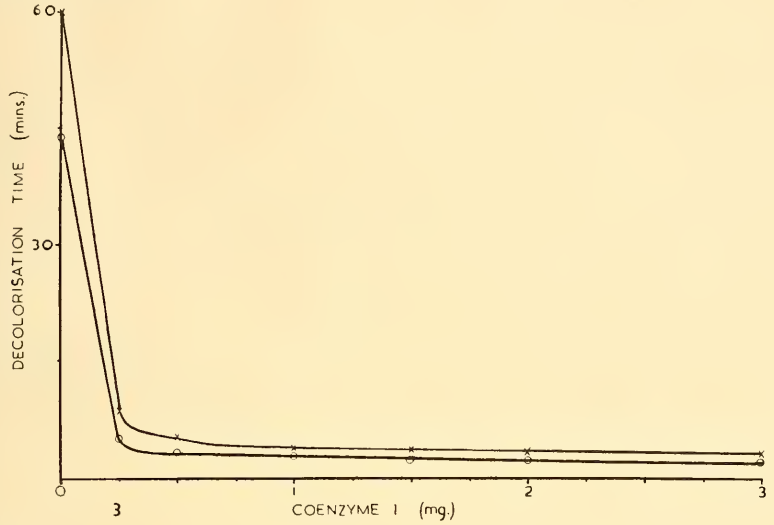
1. DISTRIBUTION OF FORMIC AND ALCOHOL DEHYDROGENASES IN THE SEEDS OF THE HIGHER PLANTS.

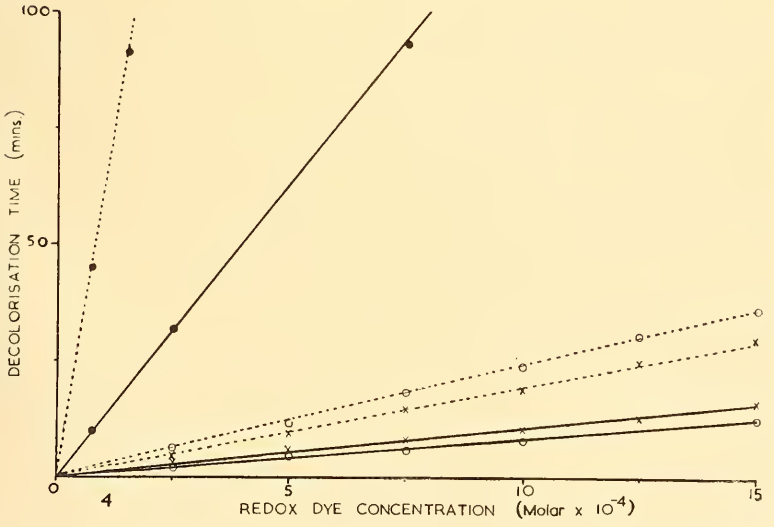
The preparations tested and the results found are contained in the following tables. The system of classification adopted is that of Engler (taken from Rendle, 1930). In the tables actual decolorization times are given but in order to simplify their study signs are also used. A negative result is denoted by the sign −, a positive result, indicating the presence of the enzyme, by +, and if the enzyme is very active (decolorization time less than half that of the control) the sign ++ is used.

On examination of the data presented in Tables 1–3 no positive evolutionary trends are apparent. However, one important fact emerges and that is that in the seeds tested,

formic dehydrogenase is relatively widespread, since the orders chosen cover an extensive range of Dicotyledons as well as some Gymnosperms and Monocotyledons.

It remained to be seen whether this widespread occurrence of the formic enzyme was purely a property of the seed. It was, therefore, decided to follow the activity of formic dehydrogenase, together with alcohol dehydrogenase, throughout the life cycle of the pea (*Pisum sativum*).





Text-figures 3, 4.

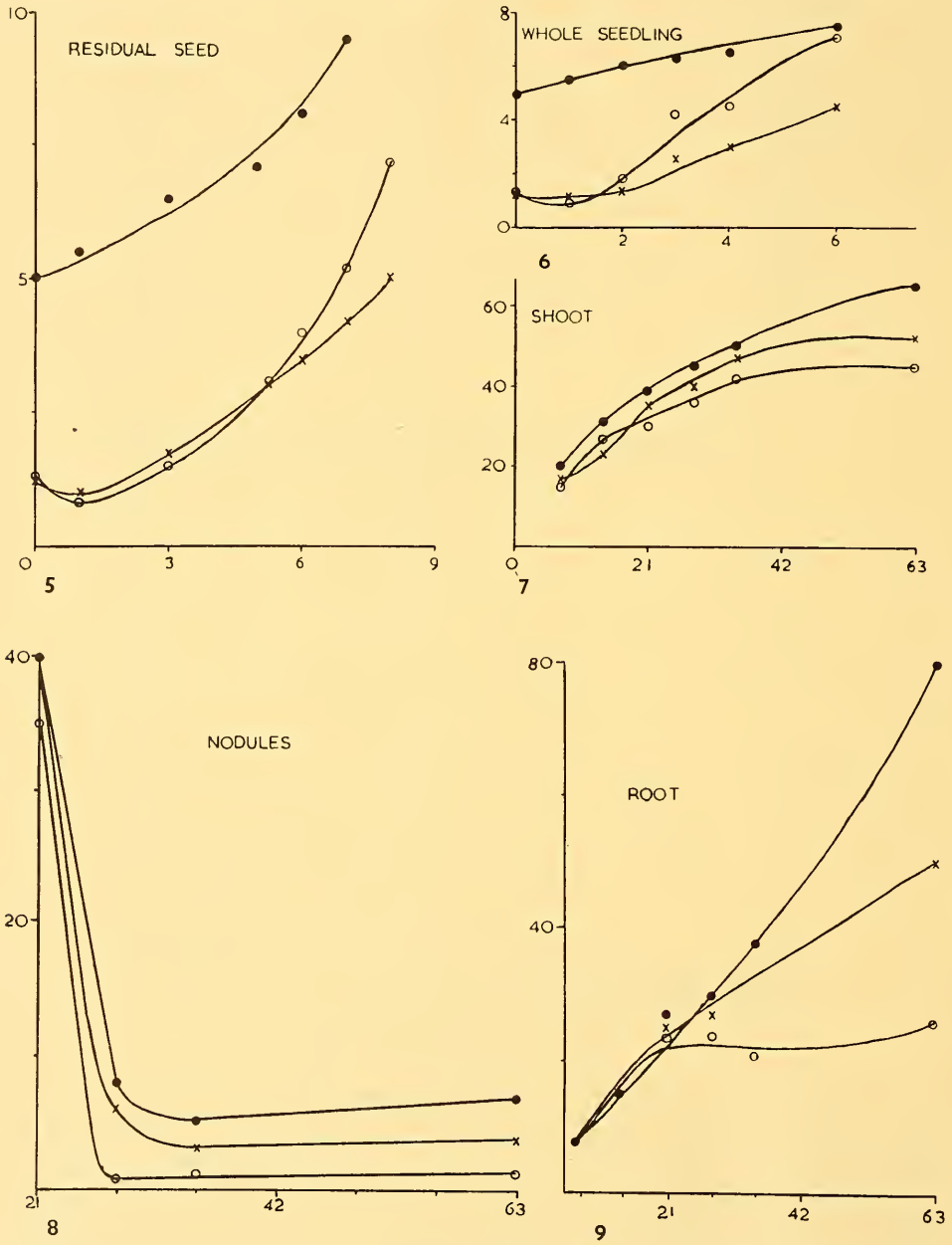
3.—Coenzyme concentration curves for the formic and alcohol dehydrogenases of peas. Thunberg contents: 0·5 ml. enzyme preparation (ammonium sulphate precipitate as in Fig. 1), 0·5 ml. buffer pH 7·0, 0·3 ml. 0·2 M sodium formate or 0·3 ml. 3·6 M ethyl alcohol, 0·2 ml. 0·025% methylene blue. Total volume 2·3 mls. x———x, formate as substrate; O———O, alcohol as substrate.

4.—Methylene blue and thionine compared as redox indicators in the formate and alcohol dehydrogenase systems. Thunberg contents: 0·5 ml. pea enzyme preparation (as in Fig. 1), 0·5 ml. 0·2 M phosphate buffer, 0·2 ml. coenzyme I solution (60μg.), 0·3 ml. 0·2 M formate or 0·3 ml. 3·6 M alcohol. The dye, in various concentrations, was placed in the hollow stopper as usual. Total volume 2·0 ml. x———x, formate as substrate; O———O, alcohol as substrate; ●———●, blank.

Dotted lines denote the decolorization when methylene blue is used; the entire lines denote the decolorization when thionine is used.

2. VARIATIONS IN FORMIC DEHYDROGENASE AND ALCOHOL DEHYDROGENASE CONTENT OF THE PEA PLANT DURING ITS LIFE CYCLE.

Seedlings up to seven days old can be grown quite readily in a dissecting dish on damp filter paper and covered by a sheet of glass. Specimens older than this were obtained from a normal garden plot. Correlation of the activity of the enzymes at the various times and from different parts of the plant were made from dry weight deter-



Text-figures 5-9.

5-9.—Variations in formic and alcohol dehydrogenase during the life cycle of the pea. x———x, formate as substrate; O———O, alcohol as substrate; ●———● blank. Decolorization time in minutes is plotted along the ordinate. the age of the plant in days along the abscissa.

minations of the water extracts used. The results are seen in Table 4. The decolorization times quoted have been corrected for a dry weight of extract of 0·03 gm. Signs have again been used to facilitate inspection of results. + indicates a decolorization time >75% of the blank but still positive for the dehydrogenase; ++, 50–75% of the control decolorization time; +++, 25–50% of the control, and ++++ <25% of the control time.

In Table 4 and Text-figures 5, 6, 7, 8 and 9 the general trends in the activity of these two dehydrogenases are apparent. Alcohol dehydrogenase decreases in activity as germination proceeds until after six days there is very little present. When the plant becomes older, however, there is a gradual increase of this enzyme, especially in the nodules and later in the root, shoot and young seed. Formic dehydrogenase similarly diminishes on germination although not as much as the alcohol enzyme. It does not reach high activity again until the seed pods begin to develop.

Finally it was necessary to discover whether these enzymes are dormant in the mature dried seed, or whether they only appear upon soaking and germination. Comparison between the dehydrogenase activity of seeds soaked overnight and the flour of seeds ground up in a coffee grinder before extracting revealed no difference.

General Conclusions.

Once again the widespread occurrence of formic dehydrogenase must be emphasized. Of the ninety-three species of plant seed examined it is of interest to note that fifty-four contained formic dehydrogenase and sixty-nine alcohol dehydrogenase.

The striking generic trend in this survey is that all of the 28 species of *Leguminosae* seeds examined contain the formic enzyme, mostly in a very active form. Some work has been carried out on the possibility of a special role for this enzyme in nitrogen fixation in the *Leguminosae* and it was shown (*a*) that not only is formate not metabolized by *Rhizobia* but that it actually inhibits the small endogenous respiration of the latter and (*b*) that the presence of the formic dehydrogenase does not depend on the nodule bacteria, since it is present whether the sterile plants are inoculated or grown without nodules.

Finally, it appears from results in Table 4 that the formic enzyme is most active in the seed. This activity is dormant in the mature dried seed, diminishes in activity with germination of the seed and growth up to the adult stages and increases in activity again in the germinating seed. Activity of the alcohol enzyme follows a similar course except that it decreases much more rapidly on germination and it is greater in the mature plant than that of formic dehydrogenase. The extremely active alcohol dehydrogenase of nodular material is worthy of special note.

Summary.

1. Ninety-three species of plant seed have been examined for the presence of formic dehydrogenase and alcohol dehydrogenase. Of these, fifty-four contained the former enzyme and sixty-nine the latter.

2. Variation in the activity of both enzymes throughout the life cycle of the pea are reported.

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